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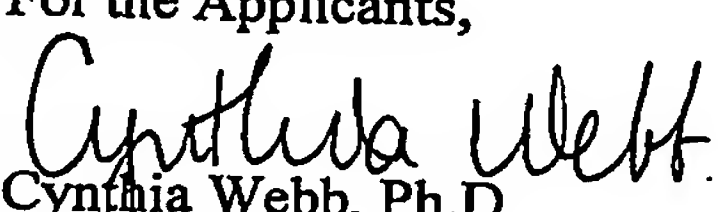
(בעברית)
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TRANSDERMAL DELIVERY SYSTEM FOR SUSTAINED RELEASE OF POLYPEPTIDES

(באנגלית)
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מבקשת פטנט from Application	לבקשה/לפטנט to Patent/Appl.	מספר/סימן Number/Mark	תאריך Date	מדינת האגוד Convention Country
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TRANSDERMAL DELIVERY SYSTEM FOR SUSTAINED RELEASE OF
POLYPEPTIDES

מערכת החדרה דרך העור לשחרור מתמשך של פוליפפטידים

TRANSDERMAL DELIVERY SYSTEM FOR SUSTAINED RELEASE OF POLYPEPTIDES

5

FIELD OF THE INVENTION

10 The present invention relates to a transdermal delivery system for sustained release of polypeptide or protein drugs and to methods of use thereof. The system comprises an apparatus that creates micro-channels in the skin of a subject in conjunction with a transdermal patch comprising at least one drug reservoir layer comprising a polymeric matrix containing the polypeptide or protein drug.

15

BACKGROUND OF THE INVENTION

20 The delivery of drugs through the skin provides many advantages: primarily, such a means of delivery is a comfortable, convenient and noninvasive way of administering drugs. The variable rates of absorption and metabolism encountered in oral treatment are avoided, and other inherent inconveniences, e.g., gastrointestinal irritation, degradation of certain drugs via gastrointestinal enzymes and the like, are eliminated as well. Transdermal drug delivery enables a high degree of control over blood concentrations of any particular drug.

25 Skin is a structurally complex, relatively thick membrane. Molecules moving from the environment into and through intact skin must first penetrate the stratum corneum. They must then penetrate the viable epidermis, the papillary dermis, and the capillary walls into the blood stream or lymph channels. To be so absorbed, molecules must overcome a different resistance to penetration in each type of tissue. Transport across the skin membrane is thus a complex phenomenon. However, it is the cells of the stratum
30 corneum, which present the primary barrier to transdermally administered drugs. The stratum corneum is a thin layer of dense, highly keratinized cells approximately 10-30 microns thick over most of the body. It is believed that the high degree of keratinization within these cells and their dense packing create a substantially impermeable barrier to drug penetration. With many drugs, the rate of permeation through the skin is extremely

low, and is particularly problematic for high molecular weight drugs such as peptides, polypeptides and proteins. Consequently, a means for enhancing the permeability of the skin is desired to effect transport of the drug into and through intact skin.

5 In order to increase the rate at which a drug penetrates through the skin, various approaches have been followed, each of which involves the use of either a physical penetration enhancer or a chemical penetration enhancer. Physical enhancement of skin permeation includes, for example, electrophoretic techniques such as iontophoresis or electroporation. The use of ultrasound (or "sonophoresis") as a physical penetration enhancer has also been studied. Chemical enhancers are compounds that are
10 administered along with the drug (or in some cases the skin may be pretreated with a chemical enhancer) in order to increase the permeability of the stratum corneum, and thereby provide for enhanced penetration of the drug through the skin. However, a major disadvantage exists when using such chemical enhancers as skin damage, irritation, and sensitization are often encountered.

15 U.S. Patent 6,148,232 to Avrahami, which is incorporated herein in its entirety by reference, describes apparatus for applying electrodes at respective points on skin of a subject and applying electrical energy between two or more of the electrodes to cause resistive heating and subsequent ablation of the stratum corneum primarily in an area intermediate the respective points. Various techniques for limiting ablation to the stratum
20 corneum are described, including spacing of the electrodes and monitoring the electrical resistance of skin between adjacent electrodes. The Device for Transdermal Drug Delivery and Analyte Extraction of the type disclosed in US 6,148,232, and various improvements to that invention including those disclosed in WO 02/085451 and WO 02/092163, are also referred to hereinafter in the specification by the term "ViaDerm".

25 It has been long appreciated that administration of a therapeutic agent in a manner that does not afford controlled release may lead to substantial oscillation of its levels, at times reaching concentrations that could be toxic or produce undesirable side effects, and at other times falling below the levels required for therapeutic efficacy. A primary goal of the use of devices and/or methods for controlled release is to produce greater control
30 over the systemic levels of therapeutic agents.

Various strategies have been developed aiming at achieving controlled release of a therapeutic agent. Release by controlled diffusion is one of these strategies. Different materials have been used to fabricate diffusion-controlled slow release devices. These materials include non-degradable polymers such as polydimethyl siloxane, ethylene-

vinyl acetate copolymers, and hydroxylalkyl methacrylates as well as degradable polymers, among them lactic/glycolic acid copolymers. Microporous membranes fabricated from ethylene-vinyl acetate copolymers have been used for release of proteins, affording a high release capacity.

5 Additional strategy for controlled release involves chemically controlled sustained release, which requires chemical cleavage from a substrate to which a therapeutic agent is immobilized, and/or biodegradation of the polymer to which the agent is immobilized. This category also includes controlled non-covalent dissociation, which relates to release
10 resulting from dissociation of an agent, which is temporarily bound to a substrate by non-covalent binding. This method is particularly well suited for controlled release of proteins or peptides, which are macromolecules capable of forming multiple non covalent ionic, hydrophobic, and/or hydrogen bonds that afford stable but not permanent attachment of proteins to a suitable substrate.

U.S. Pat. No. 5,418,222 relates to single and multiple layer collagen films for use
15 in controlled release of active ingredients, particularly of PDGF. U.S. Pat. No. 5,512,301 relates to collagen-containing sponges comprising an absorbable gelatin sponge, collagen, and an active ingredient. The collagen films in U.S. Pat. No. 5,418,222 and the collagen sponges in U.S. Pat. No. 5,512,301 provide a steady, continuous and sustained release of therapeutic agents over an extended period of time.

20 U.S. Pat. No. 5,681,568 discloses a device comprising a microporous underlayment with microcapillary pores wherein said pores are coated but not completely filled by a microskin to which a biologically active macromolecular agent is bound. Microporous underlayments comprise a polymer, and microskin comprises substances selected from collagens, fibronectins, laminins, proteoglycans, and glycosaminoglycans.
25 It is believed that such devices be useful for optimizing the delivery of macromolecules, particularly of growth factors, to a therapeutic target.

U.S. Pat. No. 6,596,293 discloses a method for preparing a drug delivery material and device comprising cross-linking of a biological polymer with a cross-linking agent and loading the cross-linked biopolymer with a bioactive agent.

30 U.S. Pat. No. 6,275,728 provides a thin film drug reservoir for an electrotransport drug delivery device comprising a hydratable, hydrophilic polymer, said film capable of forming a hydrogel when placed in contact with a hydrating liquid.

International Patent Application PCT/IL03/00903, the content of which is incorporated by reference as if set forth herein in its entirety and is assigned to the

assignee of the present application, discloses a system for transdermal delivery of a dried pharmaceutical composition comprising an apparatus for facilitating transdermal delivery of a drug through skin of a subject, said apparatus capable of generating at least one micro-channel in an area on the skin of the subject, and a patch comprising a therapeutically effective amount of the dried pharmaceutical composition. It should be noted that prior art does not provide an efficient system for transdermal delivery of hydrophilic high molecular weight proteins because these proteins can hardly diffuse through the stratum corneum and because their stability in aqueous solutions is very low. International Patent Application PCT/IL03/00903 provides, for the first time, an efficient method for transdermal delivery of hydrophilic high molecular weight proteins.

There remains an unmet need for devices and methods for sustained release of transdermally delivered hydrophilic high molecular weight medications. The advantages of this approach would be particularly striking for peptides, polypeptides, and proteins as well as for other bioactive water-soluble drugs.

SUMMARY OF THE INVENTION

The present invention provides effective system and methods for sustained and slow release of an active agent delivered transdermally.

It is an object of some aspects of the present invention to provide system and methods for ablating the skin and transdermally delivering to the pretreated skin an active agent, such system and methods achieve a sustained and slow release of the active agent into the systemic circulation.

It is an additional object of some aspects of the present invention to provide system and methods for transdermally delivering an active agent using a patch comprising one or more drug reservoir layers to which the active agent is non-covalently bound and is releasable therefrom.

It is still another object of some aspects of the present invention to provide apparatus and methods for ablating the skin and transdermally delivering an active agent using a patch comprising one or more drug reservoir layers to which the active agent is non-covalently bound and is releasable therefrom.

It is now disclosed, for the first time, that use of a patch comprising a polymeric drug reservoir layer comprising an active agent, placed on an area of the skin pretreated by an apparatus that generates micro-channels provides therapeutically effective serum

levels of the active agent for extended periods of time. According to the invention, use of the apparatus of the invention generates hydrophilic micro-channels in the stratum corneum of a subject, through which exudates diffuse into the polymeric drug reservoir layer of the patch. The exudates slowly release the active agent from the polymeric drug reservoir, thus delivering it through the micro-channels to the systemic circulation over extended periods of time. As a result, a slow and sustained release of the active agent is achieved.

It is also disclosed that use of a patch comprising a polymeric drug reservoir layer, comprising a hydrophilic high molecular weight polypeptide or protein, placed on an area of the skin pretreated by an apparatus that generates micro-channels, extends and improves the transdermal delivery of the active agent as compared to the transdermal delivery of the active agent when administered in a medical patch comprising dried or lyophilized composition comprising the active agent. Moreover, the patch according to the present invention extends the release of the active agent if the release is compared to the release of the same dose of said active agent when injected subcutaneously. The present invention, therefore, provides systems and methods for improved sustained and slow release of hydrophilic high molecular weight proteins.

It is also disclosed that a patch comprising a polymeric matrix and a therapeutically active agent, further comprising protease inhibitors, maintains the stability and activity of the therapeutically active agent throughout the transdermal delivery, thus achieving therapeutic blood concentrations of the active agent similar to those achieved if the active agent is administered by subcutaneous injection. Yet, such therapeutic blood concentrations are maintained for significantly extended periods of time.

The principles of the invention are exemplified herein below using human growth hormone (hGH), a 22 kDa protein, and human insulin. It is explicitly intended that the compositions and methods comprising the system of the invention are applicable to a wide variety of proteins, polypeptides, peptides, and water-soluble bioactive molecules including, but not limited to, various growth factors and hormones.

According to a first aspect, the system of the present invention comprises an apparatus that creates micro-channels as a means for enhancing the transdermal delivery of an agent from a skin patch subsequently placed on the skin. The term "micro-channel" as used throughout the specification and claims refers to a hydrophilic pathway generally extending from the surface of the skin through all or a significant part of the stratum corneum, through which pathway molecules can diffuse. It should be appreciated that

after micro channels have been generated in the stratum corneum, the apparatus is removed from the skin, and the active agent is delivered from a patch subsequently placed on the skin into the systemic circulation.

According to the first aspect, the system also comprises a patch comprising at least one drug reservoir layer, said drug reservoir layer comprising a polymeric matrix and a therapeutically effective amount of a peptide, polypeptide, or a protein.

Typically, the polymeric matrix may be selected from biopolymers, hydrophilic synthetic polymers, derivatives, and combinations thereof. Biopolymers that may be used according to the invention include, but are not limited to, cellulose, chitin, chitosan, alginates, collagens, gelatin, pectin, glycosaminoglycans such as, for example, heparin, chondroitin sulfate, dermatan sulfate, and heparan sulfate, proteoglycans, fibronectins, carrageenans, and laminins. In a currently preferred embodiment, the drug reservoir layer comprises a collagen.

The polymeric matrix may also be selected from hydrophilic synthetic polymers. Hydrophilic synthetic polymers that may be used according to the invention include biodegradable and non-degradable polymers such as, for example, polyglycolic acid (PGA) and polylactic acid (PLA) polymers, polypropylene oxide, polyethylene oxide, polyoxyethylene-polyoxypropylene copolymers, polyvinylalcohol, polyethylene glycol, and polyurethanes. In a currently preferred embodiment, the drug reservoir layer comprises Vigilon®, a hydrogel composed of 96% water and 4% polyethylene oxide.

In a preferred embodiment, the therapeutically active agent is a polypeptide or protein selected from the group consisting of peptides, polypeptides, proteins, growth factors, hormones, and water-soluble drugs. In currently preferred embodiments, the active therapeutic agent is human growth hormone (hGH) or human insulin. It should be appreciated that the present invention encompasses a drug reservoir layer that comprises one or more peptide, polypeptide or a protein drug.

According to the invention, exudates diffuse through the micro-channels into the drug reservoir layer and release the active agent from the polymeric matrix, thus delivering it through the micro-channels to the systemic circulation.

According to the invention, the drug reservoir layer may be in a form of a film, hydrogel, or any other form, in which the properties of the polymer relating to stability and/or ability to retain the active agent, are maintained.

Generally, the pharmaceutical composition of the invention comprising an active therapeutic agent may be mixed with the solution of the biopolymer or hydrophilic

synthetic polymer during film formation, hydrogel formation, or any other form of the polymer. Alternatively or additionally, the pharmaceutical composition comprising an active therapeutic agent may be added subsequently to the formation of the film, hydrogel, or any other form of the polymer. In currently preferred embodiments, hGH solution or human insulin solution are mixed with a collagen solution during collagen film formation.

In another embodiment, the pharmaceutical composition further comprises at least one component selected from a protease inhibitor, a stabilizer, an anti-oxidant, a buffering agent, and a preservative.

It should be appreciated that the patch according to the invention may be of any suitable geometry provided that it is adapted for stable and, optionally microbiologically controlled, aseptic or sterile, storage of the drug species prior to its use. The patch further comprises at least one of the following layers: a backing layer, an adhesive, and a rate-controlling layer.

In another aspect, the present invention provides a method for sustained transdermal delivery of a therapeutically active agent from a pharmaceutical composition comprising:

- a. generating at least one micro-channel in a region of the skin of a subject;
- b. affixing a patch to the region of skin in which micro-channels are present, the patch comprising at least one drug reservoir layer, said drug reservoir layer comprising a polymeric matrix and a therapeutically effective amount of a peptide, polypeptide, or a protein; and
- c. achieving a therapeutic blood concentration of the active agent for at least 6 hours.

In one embodiment, the polymeric matrix may be selected from biopolymers, hydrophilic synthetic polymers, derivatives, and combinations thereof. In a currently preferred embodiment, the biopolymer is a collagen.

In another embodiment, the therapeutically active agent is a polypeptide or protein selected from the group consisting of peptides, polypeptides, proteins, growth factors, hormones, and water-soluble drugs. Currently, preferred exemplary embodiments are human growth hormone and human insulin.

In a further embodiment, therapeutic blood concentrations of an active therapeutic agent are maintained for at least 6 hours. Preferably, the therapeutic blood concentrations are maintained for at least 8 hours. More preferably, the therapeutic

blood concentrations are maintained for at least 10 hours. In currently exemplary embodiments, a dose of hGH of 200 μ g transdermally administered to rats or guinea pigs in a collagen film resulted in hGH blood levels of 10-50 ng/ml for about 10 hours. It should be appreciated that similar hGH blood levels in guinea pigs were maintained for about 5 hours when the same dose of hGH was transdermally administered using the apparatus of the invention in conjunction with a printed patch, in which hGH was present as a dry composition. The system of the present invention thus provides sustained and prolonged release of an active agent.

The present invention incorporates techniques for creating micro-channels by inducing ablation of the stratum corneum, using radio frequency (RF) energy, including the apparatus referred to as ViaDerm or MicroDerm disclosed in one or more of the following: U.S. 6,148,232; US 5,983,135; WO 01/85234; US 6,597,946; US 6,611,706; WO 02/085451 and WO 02/092163; International Patent Application PCT/IL03/00903; the contents of which are incorporated by reference as if set forth herein in their entirety. It is however emphasized that although some preferred embodiments of the present invention relate to transdermal delivery obtained by ablating the skin by the aforementioned apparatus, substantially any method known in the art for generating channels in the skin of a subject may be used.

The present invention will be more fully understood from the following figures and detailed description of the preferred embodiments thereof.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows the permeation of human growth hormone (hGH; 70-90 μ g) present in a collagen film (\blacktriangle) or in a printed patch (\diamond) through porcine skin in which skin micro-channels were generated and the permeation was detected by cumulative hGH amounts in an in-vitro assay.

FIG. 2 shows the permeation of hGH (70-90 μ g) present in a collagen film (\blacktriangle) or in a printed patch (\diamond) through porcine skin in which skin micro-channels were generated and the permeation was detected by hGH transdermal flux in an in-vitro assay.

FIG. 3 shows the hGH blood levels following transdermal application of printed patch containing 150 μ g hGH (\blacklozenge) or collagen film containing 200 μ g hGH (\square) on ViaDerm treated guinea pig skin.

FIG. 4 shows hGH blood levels following transdermal application of collagen film containing 200 µg hGH on ViaDerm treated guinea pig skin (□) or rat skin (♦).

FIG. 5 shows blood glucose levels following subcutaneous administration of insulin (◇) and transdermal application of collagen film containing 0.4 IU insulin on ViaDerm treated diabetic rat skin (■).

FIG. 6 shows blood glucose levels following application of insulin patches (1.5 IU insulin) to ViaDerm treated diabetic rat skin. Collagen A-Lispro (□); collagen A-NPH (◇); collagen A-Ultra Lente (▲); and collagen B-Lispro (*).

FIG. 7 shows the permeation of human insulin present in Vigilon® hydrogel patches through porcine skin in which skin micro-channels were generated and the permeation was detected by cumulative insulin amounts in an in-vitro assay.

FIG. 8 shows the permeation of human insulin present in Vigilon® hydrogel patches through porcine skin in which skin micro-channels were generated and the permeation was detected by hGH transdermal flux in an in-vitro assay.

FIG. 9 shows blood glucose levels following application of insulin-Vigilon® hydrogel patches to ViaDerm treated diabetic rat skin. Diabetic rats were injected with insulin SC and two hours later insulin-Vigilon® hydrogel patches were applied.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides systems and methods for delivering hydrophilic active agents, particularly hydrophilic high molecular weight polypeptides or proteins through treated skin in which micro-channels have been generated.

The term "micro-channel" as used in the context of the present specification and claims refers to a hydrophilic pathway generally extending from the surface of the skin through all or a significant part of the stratum corneum and may reach into the epidermis or dermis, through which molecules can diffuse. Although some preferred embodiments of the present invention are described with respect to ablating the stratum corneum by electric current or spark generation, preferably at radio frequency (RF), substantially any method known in the art for generating channels in the skin of a subject may be used (see e.g. U.S. Pat. Nos. 5,885,211, 6,022,316, 6,142,939 6,173,202, 6,148,232 and WO 02/085451 and WO 02/092163). The term "micro-pore" is used interchangeably herein.

As the micro-channels are aqueous in nature, the system of the present invention is therefore highly suitable for delivery of hydrophilic macromolecules through the new skin environment, which is created by the ablation of the stratum corneum.

5 The terms "active agent," "drug" and "therapeutically active agent" "active ingredient" are used interchangeably herein to refer to a compound or composition of matter which, when administered to an organism (human or animal), induces a desired pharmacological and/or physiological effect by systemic action.

According to the invention, the system of the invention is suitable for transdermal delivery of peptides, polypeptides, and proteins.

10 A "peptide" refers to a polymer in which the monomers are amino acids linked together through amide bonds. "Peptides" are generally smaller than proteins, typically under 30-50 amino acids in total.

A "polypeptide" refers to a single polymer of amino acids.

15 A "protein" as used herein refers to a polymer of amino acids typically over fifty amino acids. The proteins that may be used as active agents in the present invention may be naturally occurring proteins, modified naturally occurring proteins, or chemically synthesized proteins that may or may not be identical to naturally occurring proteins.

20 A "polypeptide drug" or "protein drug" as used herein is an active agent, drug or therapeutically active agent that comprises a peptide, polypeptide or protein. Pharmacologically active derivatives, analogs and fragments of polypeptide or protein drugs are included as well.

25 In one aspect, the invention provides a system for transdermal delivery of an active therapeutic agent from a pharmaceutical composition comprising an apparatus for facilitating transdermal delivery of an active therapeutic agent through skin of a subject, said apparatus capable of generating at least one micro-channel in an area on the skin of the subject, and a patch comprising at least one drug reservoir layer, said drug reservoir layer comprising a polymeric matrix and a therapeutically effective amount of a peptide, polypeptide, or a protein.

30 Suitable active therapeutic agents for use in conjunction with the principles of the invention include peptides, polypeptides, proteins, and water-soluble drugs including, but not limited to, insulin, proinsulin, follicle stimulating hormone, insulin like growth factor-1 and insulin like growth factor-2, platelet derived growth factor, epidermal growth factor, fibroblast growth factors, nerve growth factor, colony stimulating factors, transforming growth factors, tumor necrosis factor, calcitonin, parathyroid hormone,

growth hormone, bone morphogenic protein, erythropoietin, hemopoietic growth factors and luteinizing hormone, calcitonin; glucagon; clotting factors such as factor VIIC, factor IX, tissue factor, and von Willebrand factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or
5 tissue-type plasminogen activator, including human tissue-type plasminogen activator (t-PA); bombesin; thrombin; enkephalinase; mullerian-inhibiting agent; relaxin A-chain; relaxin B-chain; prorelaxin; Dnase; inhibin; activin; vascular endothelial growth factor; receptors for hormones or growth factors; integrin; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -
10 4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6, CD proteins such as CD-3, CD-4, CD-8, and CD-19; osteoinductive factors; immunotoxins; an interferon such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs), e.g., M-CSF, GM-CSF, and G-CSF; interleukins (ILs), e.g., IL-1 to IL-10; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor; viral antigen such as, for example,
15 a portion of the AIDS envelope; transport proteins; homing receptors; addressins; regulatory proteins; antibodies; and fragments of any of the above-listed polypeptides.

According to the invention, the patch comprises at least one drug reservoir layer, in which the active therapeutic agent is imbedded or non-covalently bound. Various polymers may be used to form the drug reservoir layer and include biopolymers and
20 hydrophilic synthetic polymers.

The biopolymers, which may be used according to the invention include, but are not limited to, polysaccharides, particularly cellulose derivatives such as, for example, hydroxypropyl cellulose, carboxymethyl cellulose, and hydroxyethyl cellulose, chitin and/or chitosan, alginates; collagens; gelatin; heparin; pectin; glycosaminoglycans
25 (GAGs); proteoglycans; fibronectins; carrageenans; and laminins (see, for example, U.S. Pat. Nos. 5,418,222; 5,510,418; 5,512,301; 5,681,568; 6,596,293; 6,565,879 and references therein and Curr Pharm Biotechnol., 2003, 4(5): 283-302; Crit Rev Ther Drug Carrier Syst., 2001, 18(5): 459-501; Eur J Pharm Sci., 2001, 14(3): 201-7; Adv Drug Deliv Rev., 2001, 51 (1-3): 81-96; and Int J Pharm., 2001, 221(1-2): 1-22).

30 The drug reservoir layer can be produced from a solution of soluble collagen. Soluble collagen is collagen that has an average molecular weight of less than 400,000, preferably having a molecular weight of about 300,000. A particularly suitable soluble collagen is Vitrogen (Cohesion Technologies Inc., Palo Alto, CA). However, additional form of collagen, namely atelopeptide form of collagen, may be used as well.

Atelopeptide collagen is collagen that is free of telopeptide, which is a peptide located at one end of purified collagen often associated with immunogenicity. A solution of the telopeptide form of collagen can be converted to the atelopeptide form of collagen via hydrolysis using organic acid. One of the preferred characteristics of the soluble collagen is that it possesses a minimal amount of crosslinking, i.e., 0.5% or less.

Generally, the biopolymers have charged or highly polar groups which enable them to bind the active agents. The biopolymer may be chemically modified to change its binding affinity for a selected active agent so as to improve the binding affinity to the active agent.

Hydrophilic synthetic polymers that may be used according to the invention include biodegradable and non-degradable polymers including, but not limited to, polyglycolic acid (PGA) polymers, polylactic acid (PLA) polymers, polypropylene oxide, polyethylene oxide, polyoxyethylene-polyoxypropylene copolymers, polyvinylalcohol, polyethylene glycol, polyurethanes, for example, polyurethanes based on diisocyanate/polyglycol and glycol linkages wherein the glycol is polyethylene glycol. It should be appreciated to one skilled in the art that chemical conjugates whereby biopolymers are conjugated with hydrophilic synthetic polymers to form the drug reservoir layer are also encompassed in the present invention. For example, U.S. Pat. No. 5,510,418 discloses biocompatible conjugates comprising chemically derivatized GAGs chemically conjugated to hydrophilic synthetic polymers. The conjugate comprising a GAG covalently bound to a hydrophilic synthetic polymer may be further bound to collagen to form a three component conjugate. Additionally, the polymeric materials of the invention do not need to be cross-linked although cross-linking is possible.

Typically, the number of drug reservoir layers is determined by the desired release characteristics. Generally, more layers produce more steady and more sustained release of the active ingredient. The concentration of the active ingredient in the different layers may be varied and the thickness of the different layers need not be the same. Additionally, the drug reservoir layer may comprise one or more active therapeutic agents according to the invention so as to achieve a therapeutic effect.

The principles of the invention are exemplified herein below using a collagen film as a drug reservoir layer. U.S. Pat. No. 5,418,222, which is incorporated by reference as if set forth herein in its entirety, discloses single and multiple collagen films that are useful for sustained release delivery of pharmaceuticals. However, it should be appreciated that the drug reservoir layer may also be in a form of a hydrogel reservoir.

Hydrogels are macromolecular networks that absorb water but do not dissolve in water. That is, hydrogels contain hydrophilic functional groups that provide for water absorption, but the hydrogels are comprised of cross-linked polymers that give rise to aqueous insolubility. Generally, hydrogels are composed of hydrophilic, preferably cross-linked, polymers such as, for example, polyurethanes, polyvinyl alcohol, polyacrylic acid, dextran, cellulose, alginate, chitin, chitosan, agar, agarose, carrageenan, polyoxyethylene, a polyvinylpyrrolidone, a poly(hydroxyethyl methacrylate) (poly(HEMA)), or a copolymer or mixture thereof. In addition, any other format, which respects the polymer properties of stability and retention of the active ingredient, is also conceivable.

Typically, the drug reservoir layers, preferably in the format of films, are thin, flexible, and conformable to provide intimate contact with a body skin, are capable of hydration and also are able to release an active agent from the reservoir at rates sufficient to achieve therapeutically effective transdermal fluxes of the agent. As the apparatus of the invention creates hydrophilic micro-channels through which exudates are released, these exudates release the drug contained within the drug reservoir layer.

According to the invention, the patch may comprise one or more rate controlling layers, which are usually microporous membranes. Rate controlling layers comprise biopolymers and/or synthetic polymers that comprise the drug reservoir layer. However, in contrast to the drug reservoir layers, the rate controlling layers are devoid of the active ingredient of the invention. Representative materials useful for forming rate-controlling membranes include, but are not limited to, polyolefins such as polyethylene and polypropylene, polyamides, polyesters, ethylene-ethacrylate copolymer, ethylene-vinyl acetate copolymer, ethylene-vinyl methylacetate copolymer, ethylene-vinyl ethylacetate copolymer, ethylene-vinyl propylacetate copolymer, polyisoprene, polyacrylonitrile, ethylene-propylene copolymer, cellulose acetate and cellulose nitrate, polytetrafluoroethylene ("Teflon"), polycarbonate, polyvinylidene difluoride (PVDF), polysulfones, and the like.

The various layers contact each other by any method known in the art. One such method is to place layers adjacent to each other and apply pressure to the outer sides of the layers to force the layers together. Another method is to coat the surface of each of the layers to be contacted with a solvent, such as water, before placing the layers together. In this way, a thin portion of each surface will become soluble thereby producing adhesion upon contact. Another method is to use a known adhesive on one or

more of the contacting surfaces. Preferably, the adhesive is one that will not interfere with the release of the active ingredient from a layer.

According to the invention, a patch is used to administer the active agent, in which case the active agent is present in one or more drug reservoir layers. The drug reservoir layer may itself have adhesive properties, or may further comprise an adhesive layer attached to the drug reservoir layer. The patch may further comprise a backing layer.

The backing layer functions as the primary structural element of the transdermal system and provides the device with flexibility and, preferably, occlusivity. The material used for the backing layer should be inert and incapable of absorbing drug or any component of the pharmaceutical composition contained within the drug reservoir layer. The backing is preferably comprised of a flexible elastomeric material that serves as a protective covering to prevent loss of drug via transmission through the upper surface of the patch, and will preferably impart a degree of occlusivity to the system, such that the area of the body surface covered by the patch becomes hydrated during use. The material used for the backing layer should permit the device to follow the contours of the skin and be worn comfortably on areas of skin such as at joints or other points of flexure, that are normally subjected to mechanical strain with little or no likelihood of the device disengaging from the skin due to differences in the flexibility or resiliency of the skin and the device. Examples of materials useful for the backing layer are polyesters, polyethylene, polypropylene, polyurethanes and polyether amides.

During storage and prior to use, the patch may include a release liner. Immediately prior to use, this layer is removed so that the patch may be affixed to the skin. The release liner should be made from a drug/vehicle impermeable material, and is a disposable element, which serves only to protect the device prior to application.

To optimize desirable characteristics of a pharmaceutical composition, various additives may be optionally included in the polymeric and/or drug solution. Thus, to improve the stability of the active ingredient, a suitable stabilizing agent can be added. Suitable stabilizing agents include most sugars, preferably mannitol, lactose, sucrose, trehalose, and glucose, more preferably mannitol. In order to improve water absorption, hygroscopic additives may be added as well. To produce a pH that is compatible with a particular active ingredient being used, a suitable buffer can be used in the polymeric and/or drug solution. Suitable buffers include most of the commonly known and utilized biological buffers, preferably acetate, phosphate and citrate, more preferably acetate and phosphate. A compatible pH is one that maintains the stability of an active

ingredient optimizes its therapeutic effect or protects against its degradation. A suitable pH is generally from about 3 to about 8, preferably about 5 to about 8, and most preferably about neutral pH of from about 7.0 to about 7.5. Additionally, protease inhibitors, anti-oxidants, and preservatives, alone or in combination, may be added as well.

The pharmaceutical composition comprising an active therapeutic agent may be incorporated into the solution of the biopolymer or hydrophilic synthetic polymer during film formation, hydrogel formation, or any other form of the polymer, or the pharmaceutical composition comprising an active therapeutic agent may be added subsequently to the formation of the film, hydrogel, or other form of the polymer. In currently exemplary embodiments, hGH or human insulin as the active agents were each incorporated into a collagen solution during collagen film formation. Generally, the drug solution or the drug/polymer solution is allowed to dry after film or hydrogel formation. The drying time is varied according to the temperature of drying. A suitable temperature is from about 15°C to 37°C, and a suitable time is sufficient time so that marginal loss of solvent content is essentially zero.

The amount of therapeutically active agent in the pharmaceutical composition necessary to provide the desired amounts and concentration in the serum can be determined by methods described herein below and by methods known in the art. Thus, the concentration and the quantity of the therapeutically active agent in a pharmaceutically composition per patch can be varied independently in order to achieve a desired effect.

Devices for enhancing transdermal delivery of dried or lyophilized medication

The system of the present invention further contains an apparatus for enhancing transdermal delivery of an active agent. According to a principle of the invention the apparatus is used to generate a new skin environment through which a dried or lyophilized medication is delivered efficiently.

The term "new skin environment" as used herein, denotes a skin region created by the ablation of the stratum corneum and formation of at least one micro-channel, using the system of the present invention.

In preferred embodiment of the present invention, the apparatus for enhancing transdermal delivery of a agent using RF energy is as disclosed in U.S. Patent 6,148,232 and continuations thereto, comprising: an electrode cartridge, optionally removable,

comprising at least one electrode and a main unit wherein the main unit loaded with the electrode cartridge is also denoted herein ViaDerm.

The control unit is adapted to apply electrical energy to the electrode typically by generating current flow or one or more sparks when the electrode cartridge is in vicinity of the skin. The electrical energy in each electrode within the electrode array causes ablation of stratum corneum in an area beneath the electrode, thereby generating at least one micro-channel.

The control unit comprises circuitry which enables to control the magnitude, frequency, and/or duration of the electrical energy delivered to an electrode, in order to control current flow or spark generation, and consequently to control the dimensions and shape of the resulting micro-channel. Typically, the electrode cartridge is discarded after one use, and as such is designed for easy attachment to the main unit and subsequent detachment from the unit.

To minimize the chance of contamination of the cartridge and its associated electrodes, attachment and detachment of the cartridge is performed without the user physically touching the cartridge. Preferably, cartridges are sealed in a sterile cartridge holder, which is opened immediately prior to use, whereupon the main unit is brought in contact with a top surface of the cartridge, so as to engage a mechanism that locks the cartridge to the main unit. A simple means of unlocking and ejecting the cartridge, which does not require the user to touch the cartridge, is also provided.

Optionally the electrode cartridge may further comprise means to mark the region of the skin where micro-channels have been created, such that a medical patch can be precisely placed over the treated region of the skin. It is noted that micro-channel generation (when practiced in accordance with the techniques described in the above-cited US patents and patent applications to Avrahami et al., assigned to the assignee of the present patent application) does not generally leave any visible mark, because even the large number of micro-channels typically generated are not associated with appreciable irritation to the new skin environment.

Methods for using the system of the invention

The current invention also provides a method for sustained release of a drug using a transdermal delivery system of the invention. In general embodiments, the procedure for forming the new skin environment comprises the step of placing over the skin the apparatus for generating at least one micro-channel. Preferably, prior to generating the

micro-channels the treatment sites will be swabbed with sterile alcohol pads. Preferably, the site should be allowed to dry before treatment.

In preferred embodiments of the current invention, the type of apparatus used to generate micro-channels is disclosed in US 6,148,232 and WO 02/085451. The apparatus, containing the electrode array, is placed over the site of treatment, the array is energized by RF energy, and treatment is initiated. In principle, the ablation and generation of micro-channels is completed within seconds. The apparatus is removed after micro-channels are generated at limited depth, preferably limited to the depth of the stratum corneum and the epidermis. A patch according to the invention is attached to the new skin environment.

The present invention provides a method for sustained release of an active therapeutic agent from a pharmaceutical composition, the method comprising: generating at least one micro-channel in a region of the skin of a subject, affixing a patch to the region of skin in which the micro-channels are present, the patch comprises one or more drug reservoir layers, said drug reservoir layers comprising a polymeric matrix and a therapeutically effective amount of a peptide, polypeptide, or a protein, and achieving a therapeutically effective blood concentration of the active agent for an extended period of time.

As defined herein "therapeutically effective blood concentration" means a concentration of an active agent, which results in a therapeutic effect. According to one exemplary embodiment, the active agent is hGH. According to the invention, blood concentrations of hGH in the range of 10 ng/ml to 50 ng/ml in rats and in guinea pigs were obtained within approximately 2-4 hours for a period of about 10 hours when 200 µg hGH were administered in a collagen film. In contrast, when 200 µg hGH were administered to guinea pigs in a printed patch, similar hGH blood concentrations were obtained within 1 hour for only 5 hours (see EXAMPLE 4 herein below). Thus, the method of transdermal delivery according to the present invention provides improved sustained release of hGH.

In another exemplary embodiment, the active agent is human insulin. According to the invention, human insulin (0.4 IU) transdermally administered to diabetic rats by the system of the invention normalized blood glucose levels 2.5 hours after patch application, and such normal levels were maintained for about 9 hours (see Example 7). In contrast, subcutaneous administration of insulin at the same dose reduced blood glucose levels in diabetic rats 1 hours after injection, and the normal glucose levels were

maintained for 5 hours. Thus, transdermal delivery according to the present invention provides extended release of insulin.

The present invention thus encompasses patches comprising a protein drug such as, for example, hGH or human insulin, impregnated within a polymer, preferably biopolymer or hydrophilic polymer or a combination thereof, which achieve therapeutic blood concentrations for at least 6 hours, preferably for at least 8 hours, and more preferably for at least 10 hours. However, patches comprising a protein drug, which achieve therapeutic blood concentrations for periods of time longer than 10 hours are also contemplated.

Additionally, as therapeutic blood concentrations of polypeptides of the invention are known in the art, the period of time for achieving sustained therapeutic blood concentrations can be determined by methods described herein below or by any other method known in the art.

According to preferred embodiments of the current invention, for other applications the micro-channels may be generated separately or simultaneously with the application of a medical patch. Among the other applications, the system may include a medical patch comprising an adhesive cut-out template which is placed on the skin, and through which the cartridge is placed to treat the region of skin exposed through the template. The medication, contained within a patch according to embodiments of the present invention, is attached to the template, which is to be placed over the treated region of skin. In these applications, after removing a protective backing, the template portion of the medical patch is placed on the skin and secured by the adhesive. An electrode cartridge is then affixed to the handle, the user holds the handle so as to place the cartridge against the region of skin inside the template, and the electrodes are energized to treat the skin. Subsequently, the cartridge is discarded. A protective covering is then removed from the medicated matrix by pulling on a tab projecting from the covering, so as to concurrently lift and place the medicated matrix over the treated region of skin. It is noted that the integration of the template and the patch into a single unit assists the user in accurately placing the medicated patch onto the treated area of skin. Utilizing the system of the invention in this manner becomes advantageous for disinfected applications.

For still other applications, an integrated electrode/medicated pad cartridge is used to provide a practical apparatus as disclosed in International Patent Application WO 02/092163 which is assigned to the assignee of the present patent application and is

incorporated by reference as if set forth herein in its entirety, is also denoted MicroDerm. In these applications, the cartridge comprises an electrode array, a controlled unit and a medicated pad. Accordingly, no template is typically required. The user places the electrodes against the skin and this contact is sufficient to initiate current flow or spark formation within the electrode and the subsequent formation of micro-channels. An adhesive strip, coupled to the bottom of the medicated pad, comes in contact with and sticks to the skin when the electrodes are placed against the skin. A top cover on the medicated matrix is coupled to the electrode region of the cartridge, such that as the electrode region, fixed to the handle, is removed from the skin the top cover is pulled off the medicated pad and the pad is concurrently folded over the treated region of skin. This type of application eliminates the need for the user to touch any parts of the electrode cartridge or the medicated pad, thus substantially reducing or eliminating the likelihood of the user contaminating the apparatus.

In a preferred embodiment, current may be applied to the skin in order to ablate the stratum corneum by heating the cells. In one preferred embodiment, spark generation, cessation of spark generation, or a specific current level may be used as a form of feedback, which indicates that the desired depth has been reached and current application should be terminated. For these applications, the electrodes are preferably shaped and/or supported in a cartridge that is conducive to facilitating ablation of the stratum corneum and the epidermis to the desired depth, but not beyond that depth. Alternatively, the current may be configured so as to ablate the stratum corneum without the generation of sparks.

Generally preferred embodiments of the present invention typically incorporate methods and apparatus described in International Patent Application WO 02/092163 entitled "Monopolar and bipolar current application for transdermal drug delivery and analyte extraction," which is assigned to the assignee of the present patent application and incorporated by reference as if set forth herein in their entirety. For example, this application describes maintaining the ablating electrodes either in contact with the skin, or up to a distance of about 500 microns therefrom. The application further describes spark-induced ablation of the stratum corneum by applying a field having a frequency between about 10 kHz and 4000 kHz, preferably between about 10 kHz and 500 kHz.

Alternatively or additionally, preferred embodiments of the present invention incorporate methods and apparatus described in International Patent Application WO 02/085451 entitled "Handheld apparatus and method for transdermal drug delivery and

analyte extraction," which is incorporated by reference as if set forth herein in its entirety.

Still further alternatively or additionally, preferred embodiments of the present invention incorporate methods and apparatus described in the above-cited US Patent 6,148,232 to Avrahami, which is assigned to the assignee of the present patent application and which is incorporated by reference as if set forth herein in its entirety.

In some preferred embodiments of the present invention, the cartridge supports an array of electrodes, preferably closely-spaced electrodes, which act together to produce a high micro-channel density in an area of the skin under the cartridge. Typically, however, the overall area of micro-channels generated in the stratum corneum is small compared to the total area covered by the electrode array.

In further preferred embodiments of the present invention, a concentric electrode set is formed by employing the skin contact surface of the cartridge as a return path for the current passing from the electrode array to the skin. Preferably, the cartridge has a relatively large contact surface area with the skin, resulting in relatively low current densities in the skin near the cartridge, and thus no significant heating or substantial damage to the skin at the contact surface.

In proximity to each electrode in the electrode array, by contrast, the high-energy applied field typically induces very rapid heating and ablation of the stratum corneum.

Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present invention.

EXAMPLE 1

Preparation of hGH-collagen film based patches

Collagen used was Vitrogen 100 (3mg/ml, Cohesion Technologies Inc, Palo Alto, CA, USA). Human growth hormone source was Genotropin[®] (5.3mg/16 IU, Pharmacia and Upjohn, Stockholm, Sweden). Phosphate Buffered Saline (PBS) was obtained from Biological Industries (Kibbutz Beit Haemek, Israel).

The template of the collagen patches consisted of the following: Backing liner BLF 2080 3mm (Dow film) coated with adhesive (National starch-Duro-Tak, Cat. # 3872516), covered with perforated SIL-K silicon strip 75 mm (Loctite, Cat. # 4011), opening side of 1.4 cm², and Release liner Rexam 78CD.

Collagen (Vitrogen) solution was gently mixed with PBSx10 and NaOH 0.1M at a volume ratio of 8:1:1 for collagen: PBSx10: NaOH, respectively. The desired amount of hGH was added from a stock solution of 14 mg/ml. The collagen-GH solution at a final volume of 300-400 μ l was poured to the patch template and placed at 37°C for 30 minutes until gelation occurred. The patches were then air dried, covered with Parafilm, packed with laminate bag, silica gel and argon. The patches were kept at 4°C until used.

EXAMPLE 2

Recovery of hGH from the collagen film based patches

Human GH content in the collagen films was determined by extraction of the hGH to a PBS solution and by quantitative analysis using size exclusion HPLC. The recovery of hGH from the collagen film is summarized in Table 1.

Table 1. Recovery of hGH from collagen based patches.

Drug	Base of film	Recovery of drug (% of initial amount)
hGH	Backing liner (Dow film)	94 \pm 7
	Adhesive (Duro-Tak)	100 \pm 21

Three hundred micrograms of hGH were loaded on each patch. The recovery results are the mean \pm SD of three experiments.

As shown in Table 1, over 90% of the initial amount was extracted from the films.

There was no significant difference in the amount extracted when the collagen was poured directly to the adhesive or to a backing liner, although the variation within the adhesive group was higher.

For studying the release kinetics, the collagen films were suspended in PBS (2 ml) and incubated at room temperature. At different time points, the buffer was replaced with a fresh buffer and the amount of hGH was analyzed by size exclusion HPLC.

Collagen of the Vitrogen type released the majority of the hGH within 1 hr. However, as the amount of intracellular fluid that reaches the patch while it is being applied on the skin is small, the release through ViaDerm treated skin was next examined.

EXAMPLE 3

In vitro skin hGH permeation study

The permeability of hGH through porcine skin was measured in vitro with a Franz
5 diffusion cell system (house made). The diffusion area was 2 cm². Dermatized (300-
500 µm, Electric Dermatome, Padgett Instruments Ltd, Kansas, MI, USA) porcine skin
was excised from slaughtered white pigs (breeding of Landres and Large White, locally
grown in Kibbutz Lahav, Israel). Transepidermal water loss measurements (TEWL,
Dermalab Cortex Technology, Hadsund, Denmark) were performed and only those
10 pieces with TEWL levels less than 15 g/m² /h were mounted in the diffusion cells.

For the preparation of a printed patch with a required amount of hGH, the volume
of each droplet was calculated according to the concentration of the hGH in the solution
and accordingly the syringe's plunger displacement which is required per one droplet
printing was adjusted, wherein the range of 0.035 - 0.105 mm corresponded to 0.09 -
15 0.18 µl. This range of displacement was fed into a Basic program that controlled the
printing. Next, the Backing layer film (DOW BLF2080™, The Dow Chemical Company,
MI, USA) was placed flat with the bright side up on a flat metal block. The syringe
containing the hGH solution was loaded into the XYZ dosing machine, which then
placed measured hGH drops on the backing liner. It should be noted that within a few
20 minutes the drops started to dry consecutively. Once the 144 dots array of printed
droplets was formed the printing of new array started on a new position. According to
this procedure it was possible to form up to 6 arrays on a 5.5 x 1.6" backing liner.
Sections, 2x2 cm², of the printed 144 dotted arrays were kept at 4°C in close vials.

Skin micro channeling was performed using the ViaDerm™ instrument. The
25 density of the microelectrode array used in all the studies was 100 microelectrodes/cm².
The device was applied twice on each location, so the density of the micro channels was
200/cm². The skin was treated with an applied voltage of 330V, frequency of 100kHz,
two bursts, 700 microsec burst length, and no current limitation. Following ViaDerm
application, the TEWL was measured again to control the operation.

30 The skin pieces were washed 3 times with PBS, and hGH printed patches or hGH
collagen films were placed on the stratum corneum side. The skins plus the patches or
films were then placed on the acceptor cell with the stratum corneum facing upwards,
and the donor chambers were clamped in place. The acceptor cells were filled with
phosphate buffered saline (PBS, pH 7.4) that contained 0.1% sodium azide (Merk,

Dermstadt, Germany), 1% bovine serum albumin (Biological Industries, Kibutz Beit-Hamek, Israel) and protease inhibitors cocktail (1 tablet per 50 ml PBS, Complete Mini, Roch). Samples from the receiver solutions were collected into tubes at predetermined times for up to 20 hr period. The samples were kept at 4°C until analyzed. hGH analysis was performed by ELIZA kit (DSL-10-1900, Diagnostic Systems Laboratories, Inc. Webster, Texas, USA).

Results

The cumulative permeability of hGH in printed patches or collagen films through ViaDerm treated skin is shown in FIG. 1. As shown in FIG. 1, administration of hGH in a collagen film resulted in a lower hGH permeation compared to that obtained from a printed patch one hour after film or printed patch administration. These results indicate that a delayed delivery was achieved. Following administration of hGH in a collagen film, the cumulative hGH amount increased with time and the total permeation amount was two fold higher than the amount permeated following the application of the hGH printed patch (FIG. 1). The hGH flux values (amount permeated per cm² per hr) are shown in FIG. 2. The flux after delivery of hGH in a printed patch was 6 times higher after 1 hr in comparison to the collagen films. However, at time points from 3 hr to 20 hr the flux of the collagen film group was higher than that observed for the printed patch. The in-vitro results suggest that an extendable delivery of hGH can be attained with collagen films.

EXAMPLE 4

In vivo hGH Transdermal Delivery

Male Guinea pigs (500-800 grams, Dunkin Hartley, Harlan laboratories Ltd., Israel) and Male rats (350-400, Sprague Dawley, Harlan laboratories Ltd., Israel) were pre-medicated with IP injections of 10% ketamin/ 2% xylazine solution at a ratio of 70:30, 1 ml/kg. Anesthesia was maintained with either isofluorane or halothane gas. The abdominal skin hair was shaved carefully, and was cleaned with isopropyl alcohol. After 30 min, transepidermal water loss measurements (TEWL, Dermalab Cortex Technology, Hadsund, Denmark) were performed to check skin integrity. Skin micro channeling was performed by the use of the ViaDerm instrument with the conditions described in Example 3 herein above. TEWL was then measured again to control the operation. The treated skin was covered with the hGH printed patches or hGH collagen films and blood

samples were withdrawn from a preinserted carotid cannula in guinea pig or from the rat tail at 0, 2, 4, 6, 9, 12, and 15 hr post application.

Results

Delivery of hGH through ViaDerm treated guinea pig skin is shown in FIG. 3.

5 Embedding the hGH in collagen resulted in an extended delivery time in comparison to a printed patch. The in-vivo findings correlate with the in-vitro results. Maximal delivery was obtained after 6 hr with a collagen film and after 2 hr with a printed patch (FIG. 3). Moreover, hGH was not detected in the blood 9 hr after administration of the printed patch while significant levels were detected at that time following administration of the collagen based patches (FIG. 3).
10

Delivery of hGH through ViaDerm treated guinea pig skin and rat skin are shown in FIG. 4. As shown in FIG. 4, an extended delivery of hGH was observed also in rats. The curve profile in rat was similar to that of guinea pig.

A prolonged delivery was also achieved when higher amounts (300 µg) of hGH
15 were administered (see Table 2).

Table 2. hGH serum levels after transdermal application to ViaDerm treated guinea pig skin.

Time	hGH ng/ml	
	Collagen patch	Printed patch
6	33.08 ± 10.90	42.55±9.65
9	17.71 ± 6.78	4.90 ± 5.07
12	10*	0*
15	1.83 ± 1.60	0.22 ± 0.08

20 As shown in Table 2 similar hGH levels were observed 6 hrs after ViaDerm and patch application (33.1±10.9 and 42.6±9.7 ng/ml for collagen film based patch and printed patch, respectively, Table 2). However, much higher hGH serum levels were observed in the collagen group at later time points (17.7±6.8 versus 4.9±5.1 ng/ml for collagen film based patch and printed patch, respectively, Table 2). Thus, the in-vivo
25 results indicate that an extended release profile can be achieved by the use of biocompatible collagen films.

EXAMPLE 5

Preparation of insulin-collagen film based patches

Collagen used was either Vitrogen 100 (3mg/ml, Cohesion Technologies Inc, Palo Alto, CA, USA) or Atelocollagen (6.5%, Koken Co, LTD, Tokyo, Japan). The human recombinant insulin Humalog[®] (Lispro-100 IU/ml), Humulin N[®] (NPH-100 IU/ml), and Humulin U[®] (Ultra Lente (UL)-100 IU/ml), were purchased from Lilly (Lilly France S.A., Fegersheim, France).

Phosphate Buffered Saline (PBS) was obtained from Biological Industries (Kibbutz Beit Haemek, Israel).

10 The template of the collagen patches consisted of a backing liner (BLF 2080 3mm, Dow film) at dimensions of 2.25cm².

Collagen Vitrogen (collagen A) solution was gently mixed with PBSx10 and NaOH 0.1M at a volume ratio of 8:1:1 for collagen: PBSx10: NaOH, respectively. The desired amount of insulin was added from a stock solution of 100 IU/ml. The collagen insulin solution at a final volume of 300-320 μ l was poured to the patch template and placed at 37⁰C for 60 minutes until gelation occurred. The patches were then air dried and packed with laminate bag, silica gel and argon. The patches were kept at 4⁰C until used.

20 Cold atelocollagen (collagen B) was diluted to the desired concentration with cold PBS that contained insulin. The amount of insulin was calculated according to the final selected amount in the patch. Insulin was diluted from a stock solution of 100 IU/ml.

The atelocollagen-insulin solution at a final volume of 300 μ l was poured to the patch template and air-dried. The patches were packed and kept as described above.

25 EXAMPLE 6

Insulin content in insulin-collagen patches before and after transdermal application

ViaDerm Engineering Prototype was used. The diameter of the microelectrode array used in all the studies was 80 μ m, and the density was 75 microelectrodes/cm². Rat skin was treated with an applied voltage of 330V, frequency of 100kHz, two bursts, 700 microsecond burst length, and no current limitation.

Insulin content in all the collagen patches was determined by extraction of the insulin to a PBS or HCl 0.1N solutions (for insulin-lispro or insulin-NPH/UL, respectively) and quantitated by RF-HPLC analysis.

Results

- 5 Summary of the analysis of the insulin content in the collagen patches before and after the transdermal application is shown in Table 3.

Table 3. Insulin content within various insulin-collagen patches before and after transdermal delivery.

Group	Experiment #	Input IU of insulin	IU in patch	Residual insulin in patch following transdermal application
Vitrogen 0.3% - Insulin Lispro	T439	1.8	1.5 ± 0.1	0.6 ± 0.1
Atelocollagen 0.9% - Insulin Lispro	T443	1.8	1.4 ± 0.1	0.3 ± 0.1
Vitrogen 0.3% - Insulin NPH	T444	1.8	1.6 ± 0.1	0.6 ± 0.0
Vitrogen 0.3% - Insulin UL	T445	1.8	1.3 ± 0.0	0.6 ± 0.0
Vitrogen 0.3% - Insulin Lispro, LD	T449	0.6	0.4 ± 0.0	0.0 ± 0.0

As shown in Table 3, high dose patches (1.8 IU of insulin input) contained 1.5 IU insulin in average, which constitute 83% of the initial amount applied. Low dose (LD) patches (0.6 IU of insulin input) contained 0.4 IU insulin, which constitute 67% of initial amount applied. The 20-30 % of the initial insulin input that were not extracted were probably bound to the collagen.

It should be noted that the discrepancy in insulin content within the different collagen patches was relatively small (standard deviation of 0-7%; Table 3). The residual insulin left within a patch following insulin transdermal application correlated with the amount of insulin in the collagen patch and with the duration of application of the patch placed on the skin. The 0.4 IU patches were placed on the skin for 12.5 hr and no residual insulin was found (Table 3). The 1.5 IU patches contained almost 4 times more insulin than the LD patches and were placed on the skin for shorter period of time (8-10 hr). The residual insulin within these patches was found to be about 20% and 40% of the insulin input amount for atelocollagen patches and vitrogen patches, respectively (Table 3).

These results indicate that insulin can be transdermally delivered from LD insulin-collagen patches at a maximal efficacy (100 % of the insulin input). Such delivery is somewhat lower when insulin is delivered from insulin-collagen patches, in which the insulin content is higher (1.5 IU insulin). However, higher efficacy may be obtained if one prolongs the duration of the patch application.

EXAMPLE 7

Bioactivity of insulin embedded in collagen films in diabetic rats

Male rats (300-325, Sprague Dawley, Harlan laboratories Ltd., Israel) were deprived of food and received water ad libitum 48 hr prior to patch applications. Streptozotocin (55 mg/kg in citric buffer, 0.1M, pH 4.5; Sigma, St. Louis, MO, USA) was injected IP to the rats 24 hr prior to patch applications in order to induce diabetes.

Keto-Diastix-Glucose and Ketones urinalysis sticks, Glucometer[®], and blood glucose test strips were used (Ascensia Elite, Bayer). Blood glucose test strips (Ascensia Elite, Bayer) Norm: 75-108 mg/dl (lot no. A2G05EC072).

The rats were defined as diabetic when 24 hr following the injections the glucose levels were above 300 mg/dl, and positive urine glucose and negative urine ketones were observed. The diabetic rats were IP injected with a 10% ketamin/ 2% xylazine solution at a ratio of 70:30, 1 ml/kg. Anesthesia was maintained with either isofluorane

or halothane gas. The abdominal skin hair was shaved carefully, and was cleaned with isopropyl alcohol. After 30 min, transepidermal water loss measurements (TEWL, Dermalab Cortex Technology, Hadsund, Denmark) were performed to check skin integrity. Skin micro channeling was performed by the use of the ViaDerm instrument with the conditions described in Example 6. TEWL was then measured again to control the operation. The treated skin was covered with various insulin-collagen patches. Subcutaneous (SC) injections of 0.4 IU served as a positive control. Blood samples were obtained from the tip of the rat's tail and the level of blood glucose was determined at predetermined time points post application.

Results

Blood glucose levels following the application of insulin-collagen patches to micro-channeled skin of diabetic rats are shown in FIG. 5 and FIG. 6. As shown in FIG. 5, subcutaneous (SC) administration of 0.4 IU insulin to rats resulted in an immediate decrease in blood glucose levels. The SC effect continued for about 5 hr, and the glucose levels were raised above 200 mg/dl afterwards (FIG. 5). In contrast, transdermal application of 0.4 IU insulin in collagen patches caused an extended release of insulin, which was reflected by an extended decrease in blood glucose level that continued for about 9 hr (FIG. 5). In addition, a delay in the decrease of blood glucose level was observed when insulin-collagen patches were applied as compared to SC administration of insulin. This delay in the decrease of blood glucose level was due to the re-hydration of the collagen patches and the diffusion of insulin through the micro channels.

Administration of 1.5 IU of insulin in insulin-collagen patches to rats resulted in a decrease of more than 90% of the initial glucose level (FIG. 6) and in a hypoglycemic shock. As a result, the experiment was not continued for more than 10 hours (FIG. 6). It should be noted that the normal glucose levels in rats are in the range of 100-200 mg/dl. Thus, the optimal dose of insulin patches for studies in rats should be lower than 1.5 IU.

FIG. 6 shows the effect of various insulin-collagen patches placed on ViaDerm treated skin on blood glucose levels in diabetic rats. As shown in FIG. 6, two and a half hours following collagen A-lispro patch application, the levels of glucose decreased from about 400 mg/dl to about 200 mg/dl. The same decrease was achieved an hour later following collagen A-NPH or collagen A-Ultra Lente (UL) patch applications. This difference between collagen A-NPH or collagen A-Ultra Lente patches and collagen A-lispro patch was probably due to the different time period required for insulin to depart from the NPH and UL insulin-complex formulations. The application

of collagen B-lispro resulted in blood glucose profile similar to collagen A-NPH or collagen A-UL patches. These results indicate that a delay in insulin effect can be obtained by the use of differently complexed insulin.

The present findings indicate that various insulin-collagen patches exhibit a delayed and extended delivery of insulin as reflected by delayed and extended blood glucose profile. As a result, the biological effect of insulin was longer than that obtained after SC injection.

EXAMPLE 8

Preparation of insulin-Vigilon® based patches

Vigilon® hydrogel (C.R. BARD, Covington, GA, USA) was used for the preparation of the insulin patches. The human recombinant insulin Humulin® R (Regular-100 IU/ml), was purchased from Lilly (Lilly France S.A., Fegersheim, France). Phosphate Buffered Saline (PBS) was obtained from Biological Industries (Kibbutz Beit Haemek, Israel).

Squares of Vigilon® hydrogel sheet were cut at dimensions of 2.25cm^2 and were prehydrated with insulin solution prior to the transdermal application. It was found that incubation of a Vigilon square with insulin solution for 1 hr resulted in absorption of 0.2 ml of the insulin solution. Therefore, the Vigilon squares were incubated with insulin solutions that contained the desired final loading dose in 0.2 ml. The incubation solution volume for each patch was 2 ml. A stock solution of 100 IU/ml was diluted to the desired concentration with PBS. Thus, for example, in order to prepare 2.5 IU loaded patch, the Vigilon template was incubated for 1 hr with an insulin solution at a concentration of 12.5 IU/ml.

EXAMPLE 9

Content of insulin in insulin-Vigilon® patches

Insulin content in all the Vigilon® patches was determined by extraction of the insulin with PBS solutions and quantitative analysis by RF-HPLC.

Summary of the analysis of the content of insulin in the Vigilon® patches is shown in Table 4.

Table 4. Analysis of insulin content in various Vigilon® patches

Nominal dose (IU/Vigilon patch)	Insulin extracted (IU/Vigilon patch)	Insulin extracted (% of nominal dose)
0.25	0.15 ± 0.04	58.83 ± 14.82
0.50	0.31 ± 0.08	61.40 ± 15.72
2.50	1.88 ± 0.13	75.19 ± 5.11
5.00	4.10 ± 0.45	81.19 ± 8.89

As shown in Table 4, the amount of insulin extracted from high dose patches (2.5 and 5 IU input) was 1.9 and 4.1 IU, which are 75 and 81 % of the nominal dose, respectively. The amount of insulin extracted from low dose patches (0.25 and 0.5 IU input) was 0.15 and 0.31 IU, which are 59 and 61 % of the nominal dose, respectively. The 20-40 % of the initial insulin input that were not extracted were probably bound to insulin. The non-extractable amount of insulin was 2 times higher when lower dose of insulin was used probably because the binding of insulin to the hydrogel at such doses is higher.

EXAMPLE 10

In-vitro skin permeation study of insulin in insulin-Vigilon® patches

The permeability of insulin in Vigilon® patches through porcine skin was measured in vitro with a Franz diffusion cell system (home-made). The diffusion area was 2 cm². Dermatized (300-500um, Electric Dermatome, Padgett Instruments Ltd, Kansas, MI, USA) porcine skin was excised from slaughtered white pigs (breeding of Landres and Large White, locally grown in Kibbutz Lahav, Israel). Transepidermal water loss measurements (TEWL, Dermalab Cortex Technology, Hadsund, Denmark) were performed and only those skin pieces with TEWL levels less than 15g/m² /h were mounted in the diffusion cells.

Skin micro channeling was performed using the ViaDerm™ instrument as follows: The diameter of the microelectrode array used in all the studies was 80 μm, and the density was 75 microelectrodes/cm². The device was applied twice on each location, so the density of the micro channels was 150/cm². Rat skin was treated with an applied

voltage of 330V, frequency of 100kHz, two bursts, 700 microsecond burst length, and no current limitation.

Following ViaDerm application, the TEWL was measured again to control the operation. The skin pieces were placed on the acceptor cell with the stratum corneum facing upwards, and the donor chambers were clamped in place. The skin was washed 3 times with PBS, the PBS in the acceptor cells was replaced with PBS that contained 0.1% sodium azide (Merk, Darmstadt, Germany), 1% bovine serum albumin (Biological Industries, Kibutz Beit-Hamek, Israel) and protease inhibitors cocktail (1 tablet per 50 ml PBS, Complete Mini, Roch), and then insulin-Vigilon® patches were placed on the stratum corneum side. Samples from the receiver solutions were collected into tubes at predetermined times for up to 12 hr period. The samples were kept at -20°C until analyzed. Insulin analysis was performed by ELIZA kit (DSL-10-1600, Diagnostic Systems Laboratories, Inc. Webster, Texas, USA).

Results

The cumulative permeability of insulin in Vigilon® patches through ViaDerm treated skin is shown in FIG. 7. Following the administration of insulin, its cumulative amount increased with time and the total permeation amount was dose dependent (1.5, 2.8, 7.8, and 16.0 mU/cm² after 12 hr for 0.25, 0.5, 2.5, and 5 IU insulin in Vigilon®, respectively, see FIG. 7). The flux insulin values (amount permeated per cm² per hr) are shown in FIG. 8. The flux values also showed dose dependency. Thus, after 6 hr, for example, the values were 0.1, 0.2, 0.5, and 1.0 mUxcm²-1xhr⁻¹ for 0.25, 0.5, 2.5, and 5 IU insulin in Vigilon®, respectively (see FIG. 8).

The in-vitro results indicate that transdermal delivery of insulin can be achieved by its incorporation into a hydrogel and the use of the micro-channeling technology. The in-vitro permeation study suggests that sustained and controlled in-vivo insulin delivery may be achieved using the insulin- Vigilon® patches.

EXAMPLE 11

Bioactivity of insulin embedded in Vigilon® in diabetic rats

Male rats (300-325, Sprague Dawley, Harlan laboratories Ltd., Israel) were deprived of food and received water ad libitum 48 hr prior to patch applications. Streptozotocin (55 mg/kg in citric buffer, 0.1M, pH 4.5; Sigma, St. Louis, MO, USA) was injected IP to the rats 24 hr prior to patch applications in order to induce diabetes.

The rats were defined as diabetic when 24 hrs after the injections the glucose levels were above 300 mg/dl, and positive urine glucose and negative urine ketones were observed. The diabetic rats were premedicated with IP injections of 10% ketamin/ 2% xylazine solution at a ratio of 70:30, 1 ml/kg. Anesthesia was maintained with either isoflurane or halothane gas. The abdominal skin hair was shaved carefully, and was cleaned with isopropyl alcohol. After 30 min, transepidermal water loss measurements (TEWL, Dermalab Cortex Technology, Hadsund, Denmark) were performed to check skin integrity. Skin micro channeling was performed by the use of the ViaDerm instrument with the conditions described herein above in Example 10. TEWL was then measured again to control the operation. The treated skin was covered with various insulin patches. All the rats received SC injections of 0.1 IU insulin two hours before the patch application. SC injections of 0.1 IU without patch application served as comparative control. Glucose levels of blood drops from the tip of the rat's tail were determined using blood glucose test strips (Ascensia Elite, Bayer) at predetermined times post application.

Results

Blood glucose levels following the application of insulin-Vigilon® patches to micro-channeled skin of diabetic rats are shown in FIG. 9. Subcutaneous administration of 0.1 IU insulin resulted in an immediate decrease in glucose levels (see FIG. 9). The SC effect continued for a short time period (2 hr) and did not maintain a constant level of blood glucose. However, when insulin-Vigilon® patches were applied on the skin 2 hr after the SC injections, a controlled, constant, and sustained blood glucose profile was exhibited for 9 hr. No significant differences were observed between the 2.5 and 1.5 IU patches, probably due to overdosing and saturation of the effect.

The present findings indicate that insulin- Vigilon® patches exhibit an extended delivery of insulin as reflected by extended blood glucose profile. As a result, the biological effect of insulin was longer than that obtained after SC injection.

It will be appreciated by persons skilled in the art that the present invention is not limited by what has been particularly shown and described herein above. Rather the scope of the invention is defined by the claims that follow.

CLAIMS

1. A system for transdermal delivery of an active therapeutic agent from a pharmaceutical composition comprising: an apparatus for facilitating transdermal delivery of an active therapeutic agent through skin of a subject, said apparatus capable of generating at least one micro-channel in an area on the skin of the subject, and a patch comprising at least one drug reservoir layer, said drug reservoir layer comprising a polymeric matrix and a therapeutically effective amount of a peptide, polypeptide, or a protein.
2. The system according to claim 1 wherein the polymeric matrix is selected from the group consisting of biopolymers, hydrophilic synthetic polymers, derivatives, and combinations thereof.
3. The system according to claim 2 wherein the biopolymer is selected from the group consisting of hydroxypropyl cellulose, carboxymethyl cellulose, hydroxyethyl cellulose, carrageenans, chitin, chitosan, alginates, collagens, gelatin, pectin, glycosaminoglycans (GAGs), proteoglycans, fibronectins, and laminins.
4. The system according to claim 3 wherein the biopolymer is collagen.
5. The system according to claim 4 wherein the collagen is in a formulation of a collagen film.
6. The system according to claim 2 wherein the hydrophilic synthetic polymer is selected from the group consisting of polyglycolic acid (PGA), polylactic acid (PLA), polypropylene oxide, polyethylene oxide, polyoxyethylene-polyoxypropylene copolymers, polyvinylalcohol, polyethylene glycol, and polyurethanes.
7. The system according to claim 6 wherein the hydrophilic synthetic polymer is polyethylene oxide.

8. The system according to claim 1 wherein the active therapeutic agent is selected from the group consisting of peptides, polypeptides, proteins, growth factors, and hormones.
- 5 9. The system according to claim 8 wherein the protein is human growth hormone (hGH).
10. The system according to any of claims 5 and 9 wherein the collagen film further comprises hGH.
11. The system according to claim 8 wherein the protein is human insulin.
- 10 12. The system according to any of claims 5 and 11 wherein the collagen film further comprises human insulin.
13. The system according to any of claims 7 and 11 wherein the polyethylene oxide further comprises human insulin.
14. The system according to any of claims 1 to 13 wherein the patch further comprises at least one of the following layers: a backing layer, an adhesive, and a rate-controlling layer.
- 15 15. The system according to any of claims 1 to 14 wherein the pharmaceutical composition further comprises at least one component selected from the group consisting of a protease inhibitor, a stabilizer, an anti-oxidant, a buffering agent, and a preservative.
- 20 16. The system according to any of claims 1 to 15 wherein the apparatus comprising:
- 25 a. an electrode cartridge comprising at least one electrode; and
- b. a main unit comprising a control unit which is adapted to apply electrical energy to the electrode when the electrode is in vicinity of the skin, typically generating current flow or one or more sparks, enabling ablation of stratum corneum in an area beneath the electrode, thereby generating at least one micro-channel.

17. The system according to claim 16 wherein the electrode cartridge comprises a plurality of electrodes capable of generating a plurality of micro-channels of uniform shape and dimensions.
18. The system according to claim 17 wherein the electrical energy is of radio frequency.
19. A method for sustained transdermal delivery of an active therapeutic agent in a pharmaceutical composition comprising:
- a. generating at least one micro-channel in a region of the skin of a subject;
 - b. affixing a patch to the region of skin in which micro-channels are present, the patch comprising at least one drug reservoir layer, said drug reservoir layer comprising a polymeric matrix and a therapeutically effective amount of a peptide, polypeptide, or a protein; and
 - c. achieving a therapeutic blood concentration of the peptide, polypeptide, or protein for at least 6 hours.
20. The method according to claim 19 wherein the polymeric matrix is selected from the group consisting of biopolymers, hydrophilic synthetic polymers, and chemically derivatives thereof.
21. The method according to claim 20 wherein the biopolymer is selected from the group consisting of hydroxypropyl cellulose, carboxymethyl cellulose, hydroxyethyl cellulose, carrageenans, chitin, chitosan, alginates, collagens, gelatin, pectin, glycosaminoglycans (GAGs), proteoglycans, fibronectins, and laminins.
22. The method according to claim 21 wherein the biopolymer is collagen.
23. The method according to claim 22 wherein the collagen is in a formulation of a collagen film.
24. The method according to claim 20 wherein the hydrophilic synthetic polymer is selected from the group consisting of polypropylene oxide, polyethylene oxide, polyoxyethylene-polyoxypropylene copolymers, polyvinylalcohol, polyurethanes.

25. The method according to claim 24 wherein the hydrophilic synthetic polymer is polyethylene oxide.

26. The method according to claim 19 wherein the active therapeutic agent is selected from the group consisting of peptides, polypeptides, proteins, growth factors, and hormones.

27. The method according to claim 26 wherein the protein is hGH.

28. The method according to any of claims 23 and 27 wherein the collagen film further comprises hGH.

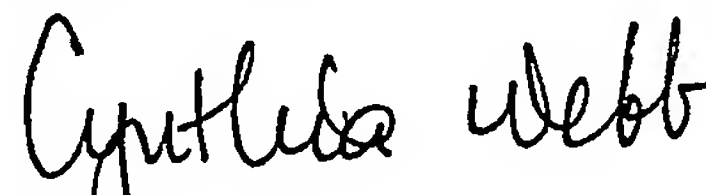
29. The method according to claim 26 wherein the protein is human insulin.

30. The method according to any of claims 23 and 29 wherein the collagen film further comprises human insulin

31. The method according to any of claims 19 to 30 wherein the patch further comprises at least one of the following layers: a backing layer, an adhesive, and a rate-controlling layer.

32. The method according to any of claims 19 to 32 wherein the pharmaceutical composition further comprises at least one component selected from the group consisting of a protease inhibitor, a stabilizer, an anti-oxidant, a buffering agent and a preservative.

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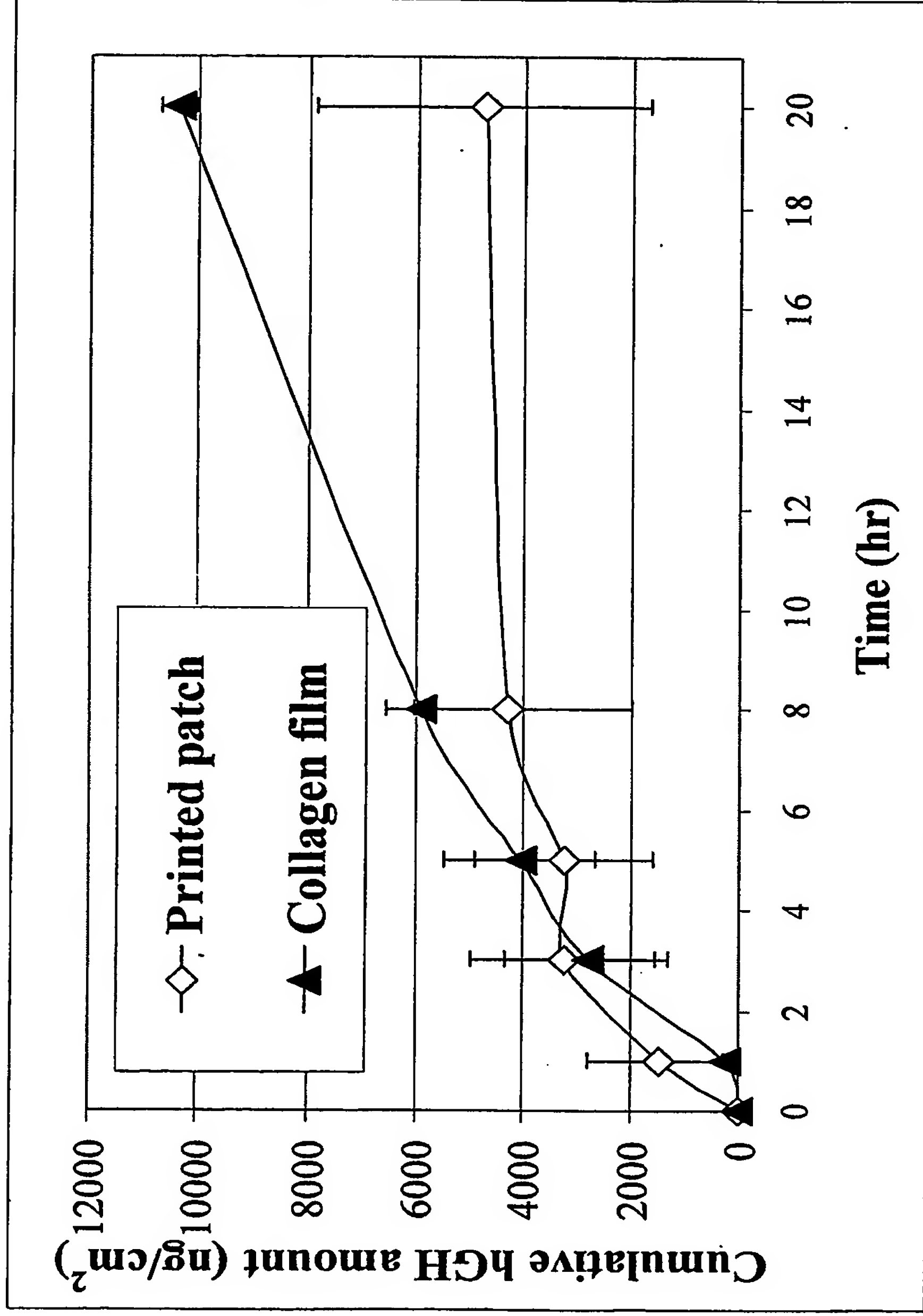


FIG. 1

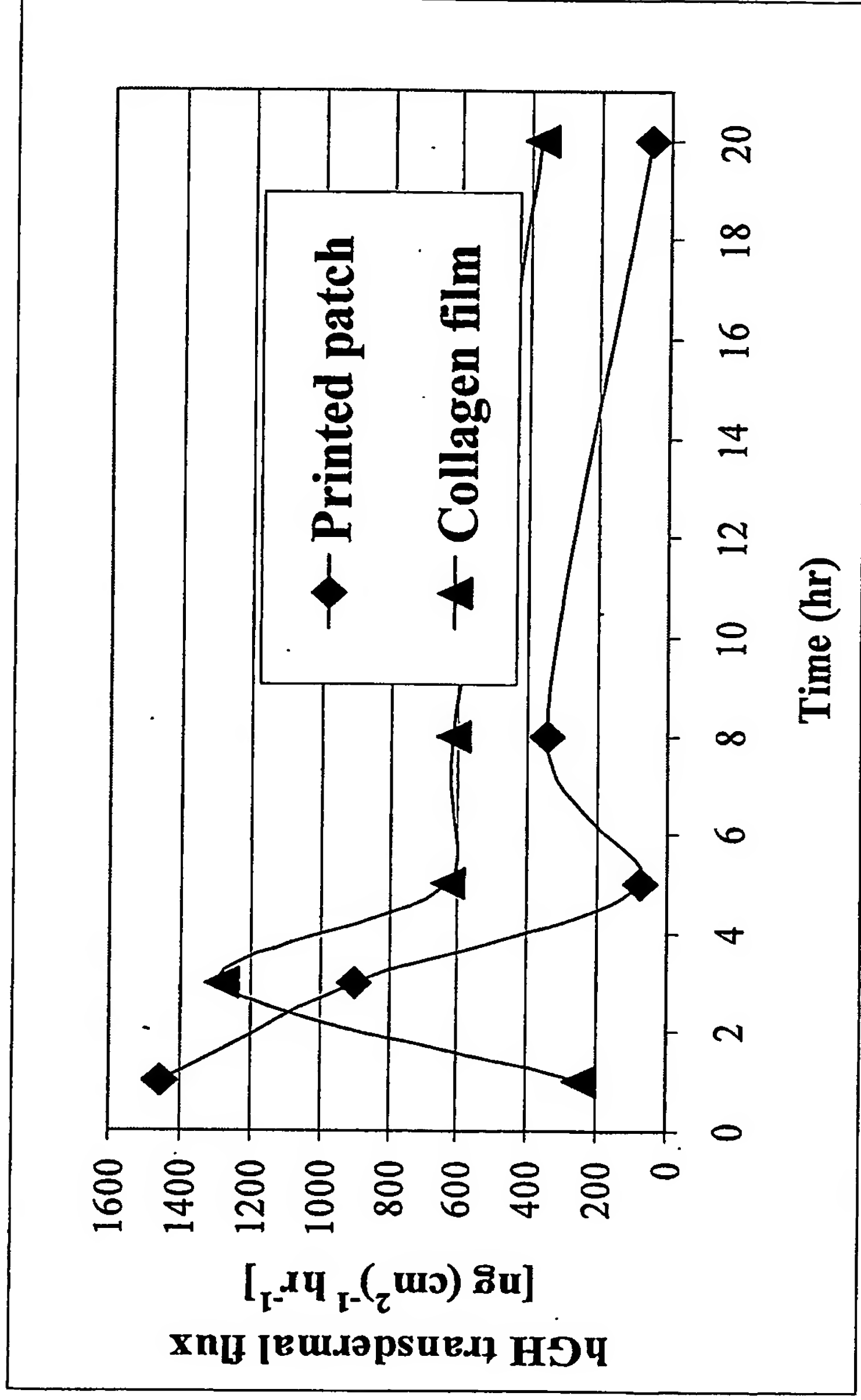


FIG. 2

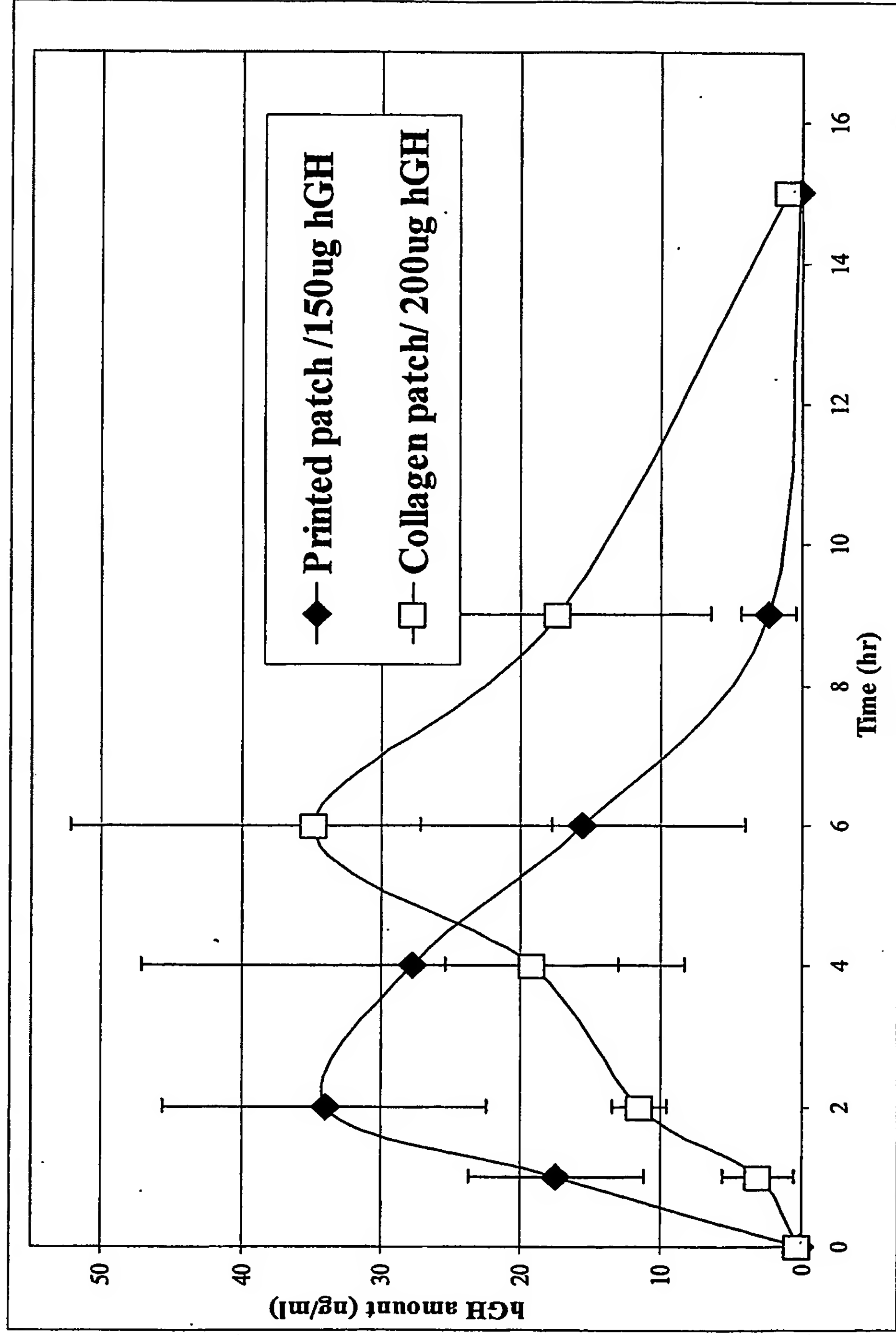


FIG. 3

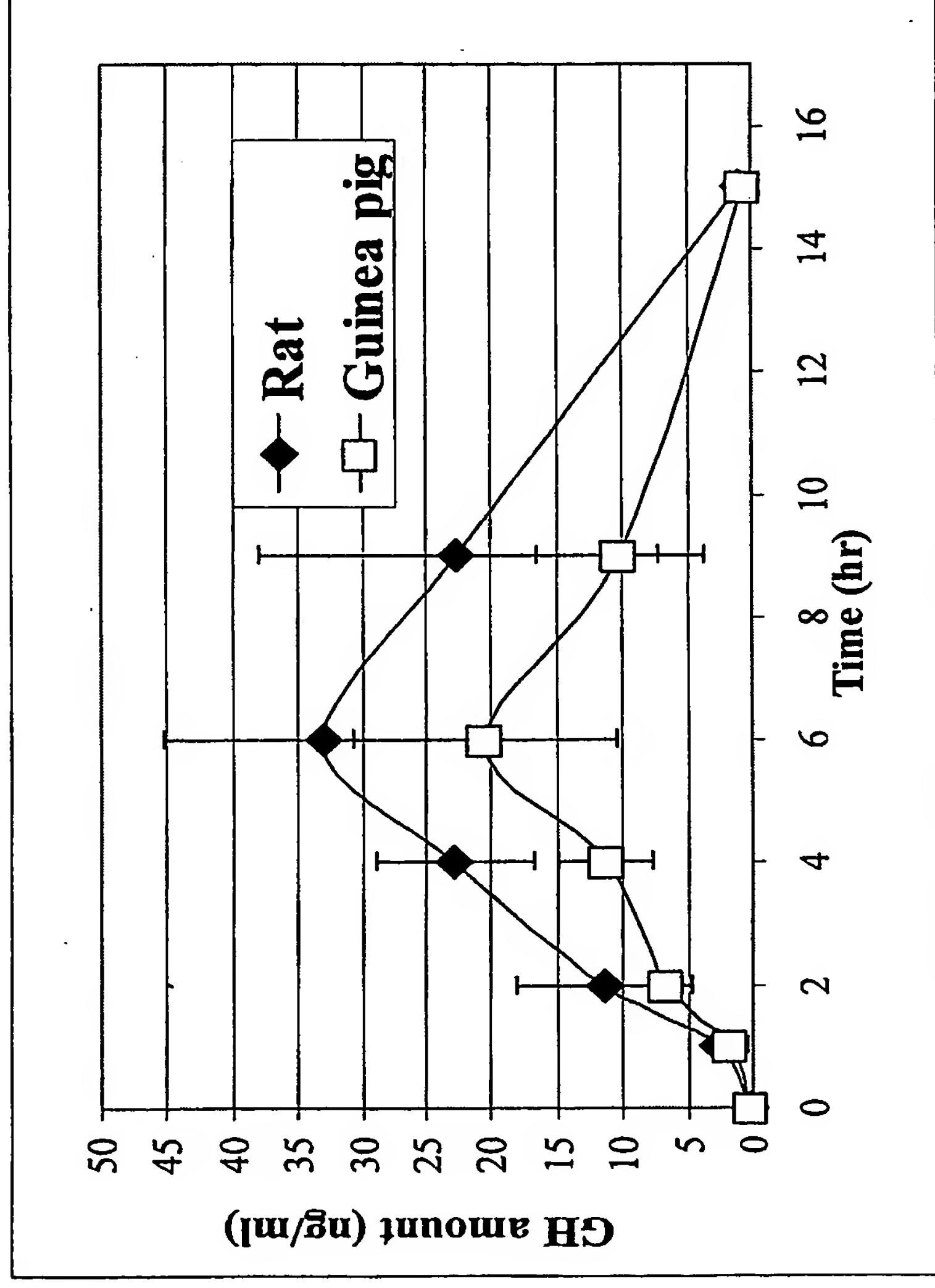


FIG. 4

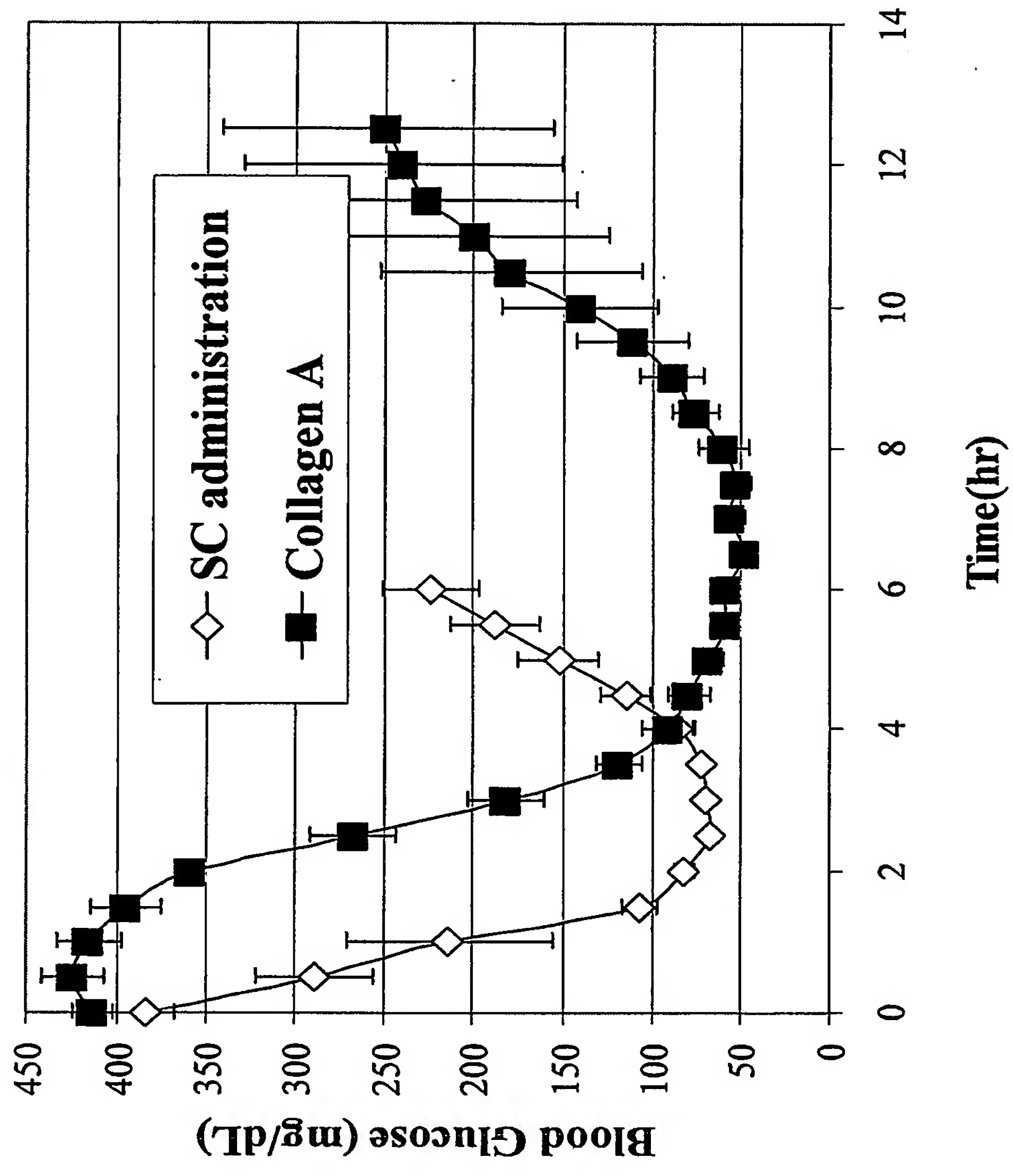


FIG. 5

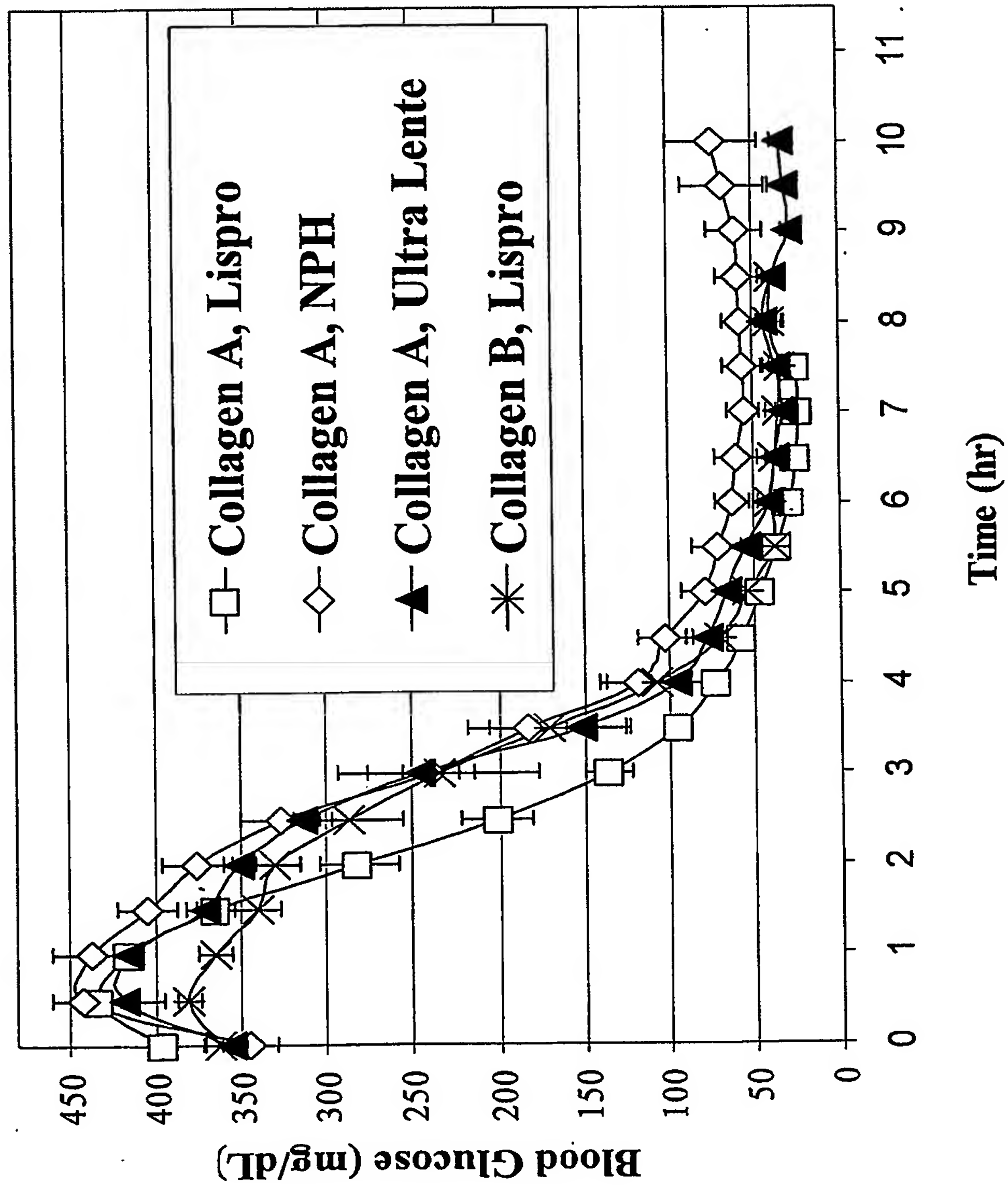


FIG. 6

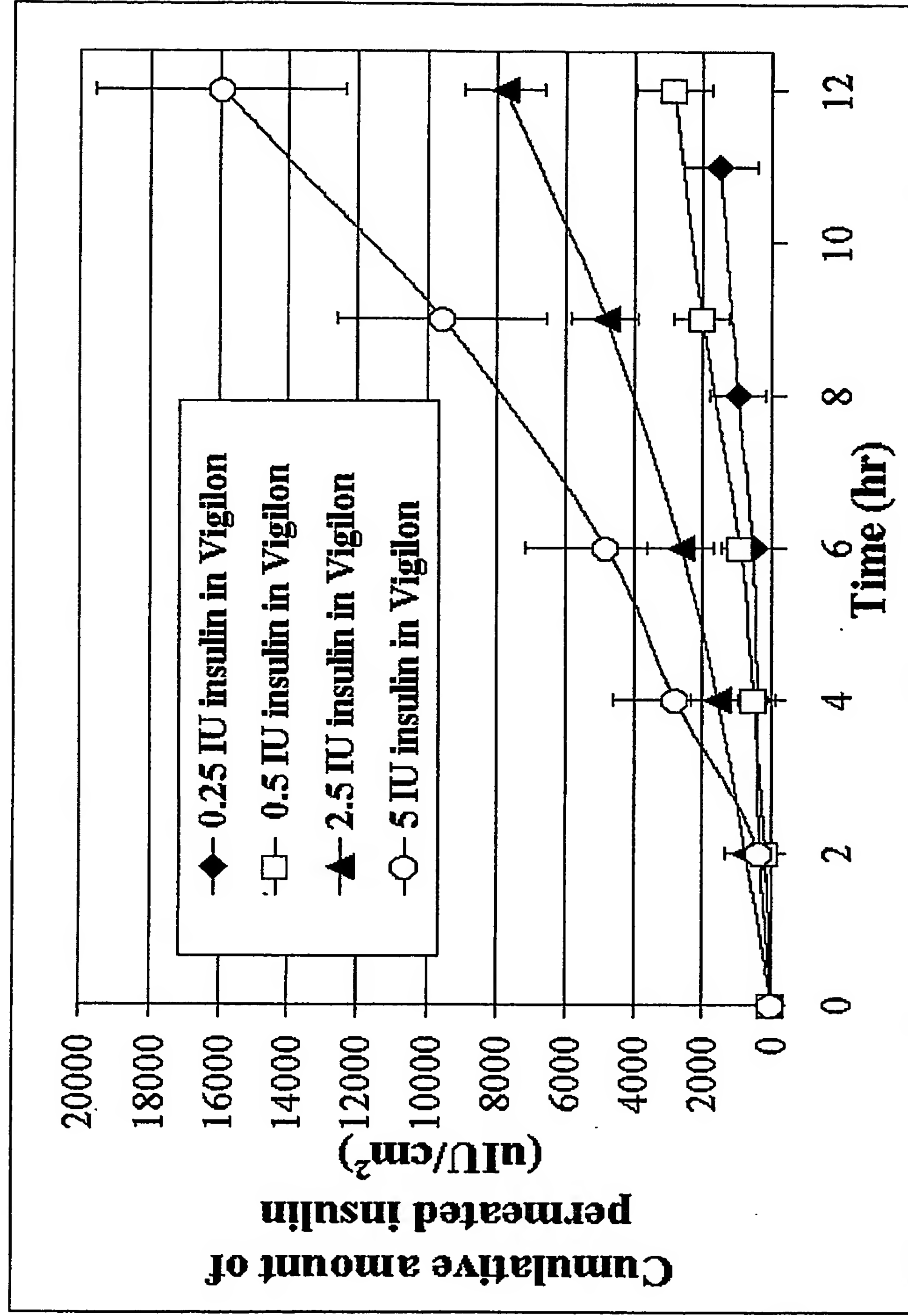


FIG. 7

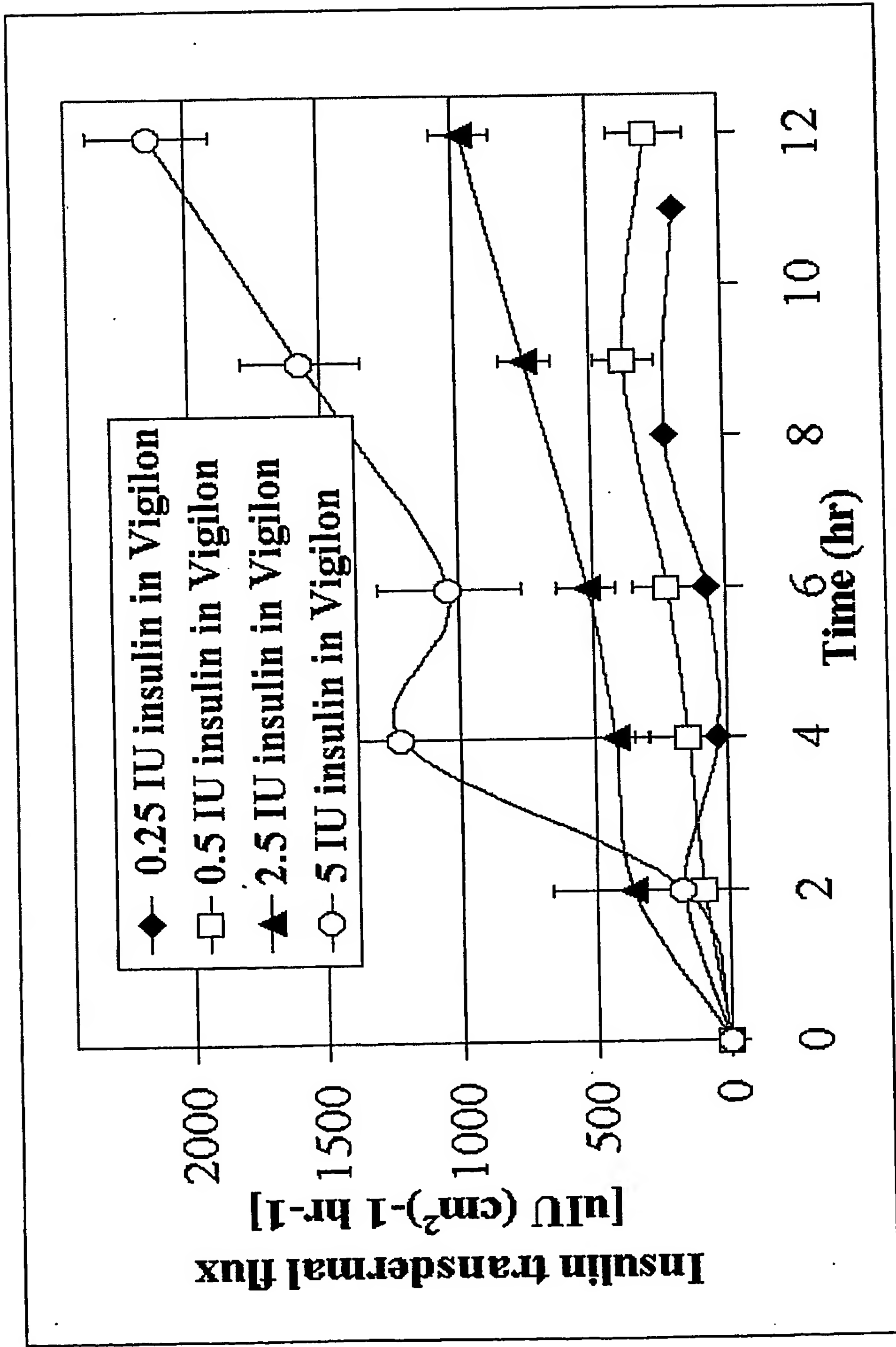


FIG. 8

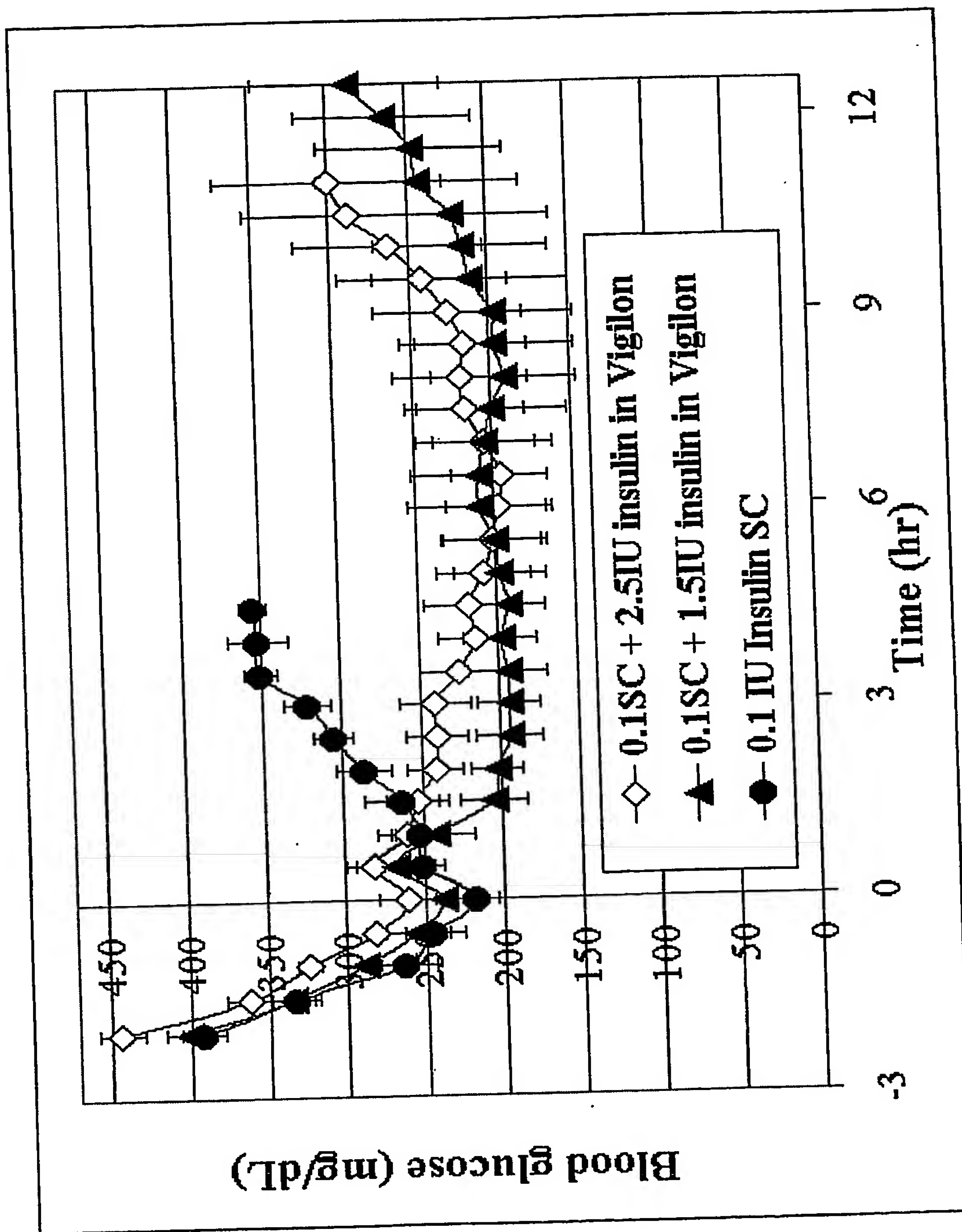


FIG. 9

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